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# *Cymbopogon distans* essential oil exhibit potent anti-candida activity by preventing the yeast-to-hyphal transition and biofilm production ability of *Candida albicans*

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Candidiasis is an infection of various part of the body involving oral cavity, vagina, penis, and skin tissue caused by an opportunistic pathogen *Candida albicans*. Candidiasis affects people of all age groups, however, infants and the old age peoples are most likely to be affected with this disease. *Cymbopogon distans* is an aromatic crop native to Himalayan region having various biological activities. The present study aimed to determine the anti-*Candida* effect of *C. distans* essential oil against *the Candida spp.* along with examining its efficacy to reduce biofilm formation and yeast-to-hyphal transitions ability of *C. albicans*. The results of the study revealed that *C. distans* essential oil has severe toxicity against all tested species of *Candida*. Moreover, the time killing kinetics data showed that the *C. distans* essential oil completely killed the *C. albicans* within the 60 min of the treatment. Additionally, a distorted morphology was observed after treatment of *C. distans* to the *C. albicans* within the short period of time. More importantly, *C. distans* essential oil inhibited the yeast-to-hyphal transition and the biofilm formation ability of *C. albicans* significantly. Thus, the data obtained from this study suggest that *C. distans* could potentially be used as therapeutic agent against *C. albicans* infection.

**Keywords:** *Candida albicans*, Candidiasis, *Cymbopogan distans* essential oil, Camphene

## INTRODUCTION

*Candida albicans* is a polymorphic fungal pathogen capable of exhibiting different morphological forms, including ovoid budding yeast, elongated pseudohyphae, and parallel-walled true hyphae. This remarkable morphological plasticity is considered one of its major virulence factors, enabling adaptation to diverse host environments and promoting tissue invasion. In addition, *C. albicans* can survive and proliferate over a wide range of pH conditions, further enhancing its pathogenic potential compared with many other fungal species[13,15].

*C. albicans* is the predominant causative agent of candidiasis, a fungal infection affecting the skin, oral cavity, gastrointestinal tract, and genitourinary tract. Among its various clinical manifestations, cutaneous candidiasis is a superficial fungal infection involving the skin and mucous membranes, including intertrigo, diaper dermatitis, erosio interdigitalis blastomycetica, perianal dermatitis, and *C. balanitis*[16]. Although several *Candida* species have been implicated in cutaneous candidiasis, *C. albicans* remains the most frequently isolated pathogen[26]. The incidence of candidiasis has increased considerably in recent years owing to the growing population of immunocompromised individuals, thereby highlighting the urgent need for the development of safer and more effective antifungal agents[16].

Several antifungal drugs have been developed for the treatment of candidiasis; however, their clinical use is often

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limited by adverse effects and the emergence of antifungal resistance. Ketoconazole, for example, has been reported to induce hypersensitivity reactions, including urticaria, and may cause adverse effects in susceptible individuals[6]. Similarly, fluconazole, an FDA-approved antifungal agent widely used for the treatment of vaginal, oropharyngeal, and esophageal candidiasis, has been associated with several adverse effects, including hepatotoxicity, anaphylaxis, fatigue, myalgia, dizziness, insomnia, neutropenia, seizures, and other systemic complications[8]. Furthermore, the extensive use of antifungal drugs has contributed to the emergence of resistant *Candida* strains, particularly against azoles and echinocandins, thereby reducing treatment efficacy and posing a major clinical challenge[21]. Therefore, there is an increasing interest in identifying novel antifungal agents from natural sources that are effective against fungal infections while exhibiting minimal toxicity and a lower likelihood of resistance development.

*Cymbopogon distans* (Nees ex Steud.) W. Watson, commonly known as Gania grass, is an aromatic perennial herb belonging to the family Poaceae and is widely distributed in the northwestern Himalayan region of India, particularly in Uttarakhand[14]. Traditionally, its essential oil has been used for the treatment of cough, cold, asthma, chronic bronchitis, and inflammatory disorders[2]. In recent years, the essential oil has also gained commercial importance in perfumery, pharmaceutical preparations, insect repellents, and other industrial products[23]. Previous phytochemical studies have demonstrated that *C. distans* essential oil contains several biologically active constituents, including geraniol, citronellal, geranyl acetate, geranial, and limonene, which contribute to its diverse biological activities[23,24,29]. However, the chemical composition of the essential oil varies considerably with geographical origin and climatic conditions, which may consequently influence its biological activity. Therefore, the present study aimed to investigate the chemical composition and anti-*Candida* activity of the essential oil isolated from wild-growing *C. distans* leaves collected from the Uttarakhand Himalayan region of India.

### Research gap

Although *C. distans* essential oil has been reported to possess antimicrobial properties, comprehensive information regarding its antifungal activity against clinically important *Candida* species, particularly its effects on fungal virulence factors such as yeast-to-hyphal transition and biofilm formation, remains limited.

### Objective

The present study aimed to characterize the chemical composition of *C. distans* essential oil by GC–MS analysis and evaluate its anti-*Candida* activity against clinically important *Candida* species. Furthermore, the study investigated its effects on fungal growth, cellular morphology, yeast-to-hyphal transition, and biofilm formation to assess its potential as a natural antifungal agent.

## Materials and Methods

### Chemicals and reagents

Yeast Extract Peptone Dextrose (YEPD) agar and YEPD broth were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Dimethyl sulfoxide (DMSO), methanol (HPLC grade), anhydrous sodium sulfate, and the antifungal drug ketoconazole were purchased from Sigma-Aldrich (Missouri, USA). Unless otherwise specified, all chemicals and reagents used in the present study were of analytical grade.

### Fungal strains and culture conditions

The fungal strains *C. albicans* (MTCC 3017), *C. parapsilosis* (MTCC 998), *C. tropicalis* (MTCC 184), *C. krusei* (MTCC 9215), and *C. glabrata* (MTCC 3982) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The cultures were maintained on YEPD agar slants at 4°C and subcultured before each experiment. Fresh colonies were inoculated into YEPD broth and incubated at 37°C for 24–48 h under aerobic conditions to obtain actively growing cultures for subsequent experiments.

### Plant material and essential oil isolation

Fresh leaves of *C. distans* were collected from the experimental fields of the Centre for Aromatic Plants, Dehradun, Uttarakhand, India (30.36338°N Latitude, 77.85008°E Longitude). The collected leaves were shade-dried and subjected to hydrodistillation using a Clevenger-type apparatus for 4 h to obtain the essential oil. The extracted oil was dehydrated over anhydrous sodium sulfate and stored in airtight amber glass vials at 4°C until further use[11,14].

### Gas chromatography–mass spectrometry (GC–MS) analysis

GC analysis were carried out by an Agilent Technologies 8890 gas chromatograph data handling system equipped with a split injector (split ratio 1:20) and fitted with FID using N2 as the carrier gas. The column was HP-5 capillary column (30m × 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60°C (hold: 2 min) programmed at a rate of 3°C /min to a final temperature of 220°C (hold: 5 min). Temperatures of the injector and detector were maintained at 250°C. The injection volume was 1.0 µL. The GC-MS analyses of the oils were performed with Agilent Technologies 8890 gas chromatograph with

7010B triple quadrupole mass detector operating in the $EI^+$ mode and equipped with a split injector (split ratio 1:10) data handling system. The column was HP-5MS capillary columns (30 m × 0.25 mm, 0.25 µm film thickness). Helium was the carrier gas at a flow rate 0.8 mL/min. Temperature program used was the same as described above for GC analysis. The temperatures of the injector (210°C), transfer line (280°C) and ion source were maintained at (230°C, 150°C, 150°C). Mass spectra was taken over *m/z* 35-450 amu that revealed the total ion current, using an ionizing voltage of 70 eV. Identification of volatile constituents was based on retention indices calculated relative to a homologous series of *n*-alkanes ($C_8$–$C_{24}$), comparison with authentic standards, and matching of mass spectra with the NIST/Wiley spectral libraries and published literature[14,23]. Quantitative analysis was performed using peak-area normalization without correction factors.

## Antifungal activity

*Agar well diffusion assay*

The antifungal activity of *C. distans* essential oil against *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata* was evaluated using the agar well diffusion method. Briefly, 100 µL of freshly prepared fungal suspension was uniformly spread onto YEPD agar plates. Sterile wells (6 mm diameter) were prepared using a cork borer, and 50 µL of essential oil at different concentrations (1–20%, v/v) prepared in 1% methanol was added to each well. Wells containing 1% methanol served as the negative control, whereas ketoconazole was used as the positive control. Plates were incubated at 37°C for 24 h, after which the diameter of the inhibition zone was measured in millimetres.

*Minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) of *C. distans* essential oil against the tested *Candida* species was determined using the broth microdilution method. Serial two-fold dilutions of the essential oil were prepared in sterile 96-well microplates. Equal volumes (100 µL) of fungal suspension were added to each well, and the plates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of essential oil that completely inhibited visible fungal growth[10].

*Minimum fungicidal concentration (MFC)*

The minimum fungicidal concentration (MFC) was determined following MIC analysis. Aliquots (10 µl) from wells showing no visible growth were subcultured onto YEPD agar plates and incubated at 37°C for 24 h. The lowest concentration that produced no visible colony growth was recorded as the MFC[7].

*Time-kill kinetics assay*

Time-kill kinetics were evaluated using the MFC of *C. distans* essential oil. Fresh fungal cultures were exposed to the MFC concentration and incubated at 37°C. Samples were collected at predetermined intervals (0–60 min), serially diluted, plated on YEPD agar, and incubated for 24 h. Colony-forming units (CFU) were counted, and fungal survival was expressed as CFU/mL[7].

*Morphological examination*

Morphological alterations induced by *C. distans* essential oil were examined using phase-contrast microscopy. Fresh cultures of *C. albicans* were treated with the MFC concentration of the essential oil for 30 min, while untreated cultures served as controls. Subsequently, 10 µL of each suspension was placed on a clean glass slide, covered with a coverslip, and examined under an Olympus CKX53 phase-contrast microscope (Olympus, Tokyo, Japan).

*Germ tube inhibition assay*

The inhibitory effect of *C. distans* essential oil on germ tube formation was evaluated according to the method described by Alves *et al*.[1] with slight modifications. Fresh colonies of *C. albicans* were inoculated into YEPD broth supplemented with 50% fetal bovine serum (FBS) in the presence or absence of essential oil and incubated at 37°C for 3 h. Germ tube formation was observed under a phase-contrast microscope, and representative images were captured.

*Cell viability assay (MTT assay)*

The effect of *C. distans* essential oil on fungal cell viability was evaluated using the MTT reduction assay[17]. *C. albicans* ($2.5 \times 10^5$ CFU/mL) was treated with MIC, MFC, 2×MIC, and 2×MFC concentrations of the essential oil for 24 and 48 h. After incubation, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 h. Formazan crystals were dissolved in 50 µL DMSO, and absorbance was measured at 600 nm using a Victor Nivo multimode microplate reader (PerkinElmer, USA).

*Biofilm formation and quantification assay*

Biofilm inhibition was evaluated according to Rangel *et al*.[22] with minor modifications. Briefly, *C. albicans* suspension ($2.5 \times 10^5$ CFU $mL^{-1}$) prepared in YEPD broth supplemented with 2% sucrose was transferred to sterile flat-bottom 96-well microplates. The essential oil was added at MIC, MFC, 2×MIC, and 2×MFC concentrations, followed by incubation at 37°C for 24 and 48 h. The wells were gently washed twice with phosphate-buffered saline (PBS), air-dried, stained with 0.4% crystal violet for 30 min, and washed three times with PBS. The bound dye was solubilized using absolute ethanol, and absorbance was measured at 600 nm. Biofilm inhibition was calculated

relative to the untreated control, which was considered 100% biofilm formation.

### Statistical analysis

All the samples were analysed in triplicate. Experimental results are depicted as the mean ± standard error of the mean (SEM). Statistical significance was calculated using the Students t-tests. * represents $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$.

## Results

### Chemical composition of *C. distans* essential oil

The chemical composition of *C. distans* essential oil was characterized by GC–MS analysis. Among the identified compounds, camphene was the predominant constituent (25.40%), followed by D-limonene (17.95%), bornyl acetate (12.47%), neryl acetate (6.95%), α-pinene (6.68%), tricyclene (3.72%), and *p*-menth-1-en-8-ol (2.21%). The complete chemical composition of the essential oil is presented in Table 1, while the representative GC–MS chromatogram is shown in Fig. 1.

### Antifungal activity of *C. distans* essential oil against *Candida* species

The antifungal activity of *C. distans* essential oil was initially evaluated using the agar well diffusion assay against five clinically important *Candida* species. The essential oil exhibited concentration-dependent antifungal activity against all tested isolates.

Among the tested species, *C. albicans* was the most susceptible, producing inhibition zones of 16 ± 1.5, 19 ± 1.0, 24 ± 1.5, and 29 ± 1.5 mm at essential oil concentrations of 1, 2.5, 5, and 10% (v/v), respectively. Similarly, inhibition zones of 15 ± 1.5, 16 ± 1.0, 17 ± 0.5, and 18 ± 1.0 mm were observed against *C. parapsilosis*, whereas *C. tropicalis* showed inhibition zones of 13 ± 2.0, 14 ± 1.5, 16 ± 1.8, and 16 ± 2.5 mm at the corresponding concentrations (Table 2). Likewise, *C. krusei* exhibited inhibition zones ranging from 12 ± 0.8 to 15 ± 0.6 mm, while *C. glabrata* demonstrated inhibition zones between 13 ± 2.0 and 17 ± 2.6 mm. Overall, the inhibitory effect of *C. distans* essential oil increased with increasing concentration, with *C. albicans* showing the highest susceptibility among the tested *Candida* species (Table 2).

### MIC, MFC and time-kill kinetics

The antifungal potency of *C. distans* essential oil was further evaluated by determining its MIC and MFC values against the tested *Candida* species. As summarized in Table 3, the MIC values were 250 µg/mL for *C. parapsilosis*, 350 µg/mL for *C. tropicalis*, 250 µg/mL for *C. krusei*, 500 µg/mL for *C. glabrata*, and 750 µg/mL for *C. albicans*.

The corresponding MFC values were 625 µg/mL, 1000 µg/mL, 750 µg/mL, 750 µg/mL, and 1000 µg/mL against *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. albicans*, respectively (Table 3).

To investigate the fungicidal kinetics of *C. distans* essential oil, a time-kill assay was performed using the respective MFC concentrations. Treatment with the essential oil resulted in a rapid reduction in fungal viability in all tested species. A decline of approximately 4 log CFU/mL was observed within 10 min of treatment in *C. albicans*, whereas a comparable reduction was achieved after 60 min in *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata*. Complete killing of *C. albicans* was achieved within 60 min of treatment (Fig. 2). In contrast, complete fungicidal activity against *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata* was observed after 120 min, demonstrating the rapid fungicidal activity of *C. distans* essential oil against clinically relevant *Candida* species (Fig. 2).

### Effect of *C. distans* essential oil on the morphology of *C. albicans*

The effect of *C. distans* essential oil on the cellular morphology of *C. albicans* was examined using phase-contrast microscopy following treatment with the MFC concentration of the essential oil. As shown in Fig. 3, untreated control cells exhibited normal oval-shaped morphology with actively budding yeast cells, indicating healthy growth. In contrast, treatment with *C. distans* essential oil caused pronounced morphological alterations characterized by disrupted cellular architecture, reduced cell density, and the presence of abundant cellular debris. Furthermore, the number of intact fungal cells was markedly lower in the treated group than in the untreated control, suggesting severe cellular damage induced by the essential oil.

### Effect of *C. distans* essential oil on the yeast-to-hyphal transition of *C. albicans*

The inhibitory effect of *C. distans* essential oil on the yeast-to-hyphal transition of *C. albicans* was evaluated using the germ tube assay. Since germ tube formation is initiated within the first few hours under inducing conditions, morphological observations were recorded at 30 min and 3 h after incubation.

As depicted in Fig. 4, untreated *C. albicans* cells initiated budding within 30 min and subsequently underwent complete germ tube and hyphal formation after 3 h. In contrast, cells treated with *C. distans* essential oil (750 µg/mL) retained their normal yeast morphology throughout the experimental period without visible germ tube or hyphal development. These findings indicate that *C. distans* essential oil effectively inhibits the yeast-to-hyphal transition, one of the major virulence determinants of *C. albicans*.

**Table 1.** Chemical composition of *Cymbopogan distans* essential oil analyzed by GC-MS

| S. No. | Constituents | RI[(a)] | RI [(b)] | Relative area (%)[(c)] | Type | Identification mode |
|---|---|---|---|---|---|---|
| 1 | Tricyclene | 921 | 930 | 3.72 | MH | d, e |
| 2 | α-Pinene | 932 | 939 | 6.68 | MH | d, e, f |
| 3 | Camphene | 946 | 953 | 25.40 | MH | d, e, f |
| 4 | Benzaldehyde | 952 | 946 | 0.62 | OC | d, e |
| 5 | Sabinene | 969 | 973 | 0.26 | MH | d, e |
| 6 | β-Pinene | 974 | 965 | 0.17 | MH | d, e |
| 7 | β-Myrcene | 988 | 991 | 1.23 | MH | d, e |
| 8 | α-Phellandrene | 1002 | 1006 | 0.44 | MH | d, e |
| 9 | Cymene | 1020 | 1025 | 0.57 | MH | d, e |
| 10 | D-Limonene | 1024 | 1027 | 17.95 | MH | d, e, f |
| 11 | 1,8-Cineole | 1026 | 1011 | 0.26 | MO | d, e |
| 12 | β-Ocimene | 1032 | 1034 | 0.14 | MH | d, e |
| 13 | γ-Terpinene | 1054 | 1055 | 0.15 | MH | d, e |
| 14 | α-Terpinolene | 1086 | 1092 | 1.07 | MH | d, e |
| 15 | Linalool | 1095 | 1098 | 0.20 | MO | d, e, f |
| 16 | *trans*-p-Menth-2-en-1-ol | 1136 | 1128 | 0.94 | MO | d, e |
| 17 | Camphor | 1141 | 1150 | 1.52 | MO | d, e, f |
| 18 | Camphene hydrate | 1145 | 1132 | 1.00 | MO | d, e |
| 19 | Borneol | 1165 | 1172 | 2.03 | MO | d, e |
| 20 | 4-Terpineol | 1174 | 1175 | 0.92 | MO | d, e |
| 21 | *p*-Cymen-8-ol | 1179 | 1183 | 0.24 | MO | d, e |
| 22 | α-Terpineol | 1186 | 1192 | 2.21 | MO | d, e |
| 23 | *cis*-Piperitol | 1195 | 1193 | 0.49 | MO | d, e |
| 24 | *trans*-Piperitol | 1207 | 1187 | 0.66 | MO | d, e |
| 25 | *trans*-Carveol | 1215 | 1217 | 0.12 | MO | d, e |
| 26 | *cis*-Nerol | 1227 | 1238 | 0.52 | MO | d, e |
| 27 | Carvone | 1239 | 1225 | 0.11 | MO | d, e |
| 28 | Piperitone | 1249 | 1248 | 0.28 | MO | d, e |
| 29 | Bornyl acetate | 1284 | 1285 | 12.47 | MO | d, e, f |
| 30 | Neryl acetate | 1359 | 1282 | 6.95 | MO | d, e, f |
| 31 | *trans*-Caryophyllene | 1408 | 1417 | 0.96 | SH | d, e |
| 32 | Linalyl butyrate | 1421 | 1459 | 0.71 | MO | d, e |
| 33 | Elemol | 1548 | 1529 | 0.24 | SO | d, e |
| 34 | Caryophyllene oxide | 1582 | 1564 | 0.26 | SO | d, e |
| 35 | γ-Eudesmol | 1630 | 1629 | 0.24 | SO | d, e |
| 36 | β-Eudesmol | 1649 | 1649 | 0.21 | SO | d, e |
| 37 | α-Eudesmol | 1652 | 1652 | 0.12 | SO | d, e |
| | Monoterpene hydrocarbon (MH) | | 57.78 | | | |
| | Oxygenated monoterpene (MO) | | 31.63 | | | |
| | Oxygenated compounds (OC) | | 0.62 | | | |
| | Sesquiterpene hydrocarbon (SH) | | 0.96 | | | |
| | Oxygenated sesquiterpene (SO) | | 1.07 | | | |
| | Total identified (%) | | 92.06 | | | |

RI, Retention Indices a) Experimental linear program retention indices determined on the DB-5MS (30 m) column; b) Literature based linear program retention indices determined on the DB-5MS (30 m) column; c) % of relative GC peak area; d) Tentative identification based on retention indices; e) Tentative identification based on mass spectra (EI, 70 eV); f) Identification based on the use of standard compounds

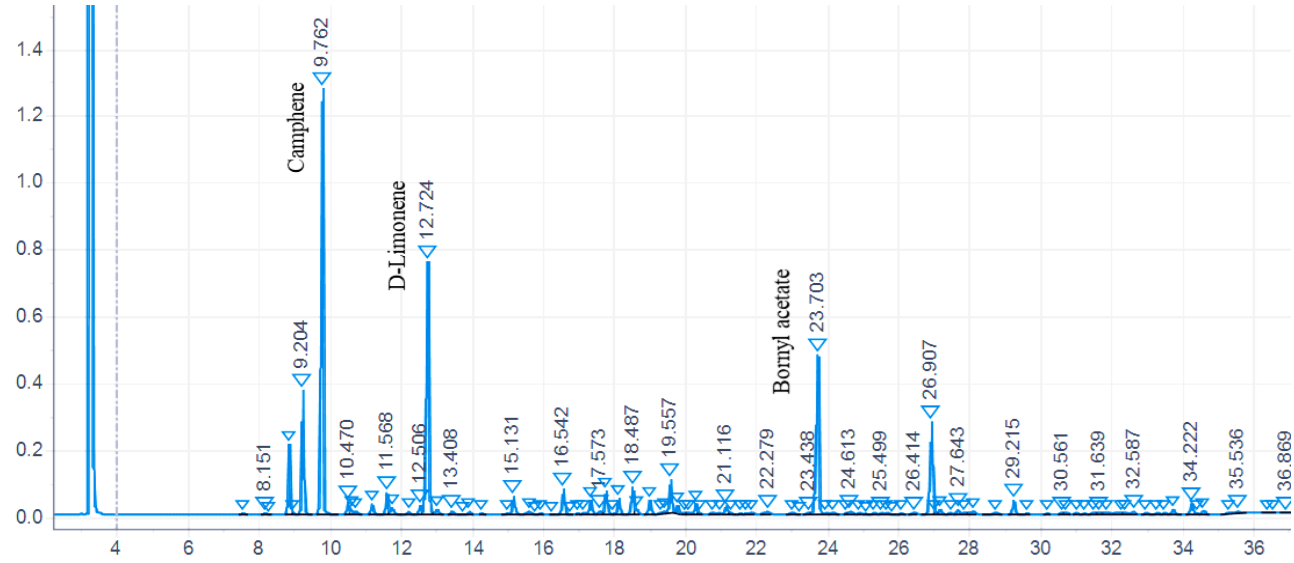


**Figure 1.** Chemical profiling of *C. distans* essential oil analysed by GC-MS

**Table 2.** Zone of inhibition (ZOI) of *C. distans* essential oil against *Candida* species. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM)

| Candida species | Concentrations (mg/mL) | | | |
|---|---|---|---|---|
| | 1 | 2.5 | 5 | 10 |
| | Zone of inhibition (mm) | | | |
| *C. parapsilosis* | 15±1.5 | 16±1.0 | 17±0.5 | 18±1.0 |
| *C. tropicalis* | 13±2.0 | 14±1.5 | 16±1.8 | 16±2.5 |
| *C. kruseii* | 12±0.8 | 13±1.3 | 14±2.1 | 15±0.6 |
| *C. glabraata* | 13±2.0 | 13±1.3 | 15±1.5 | 17±2.6 |
| *C. albicans* | 16±1.5 | 19±1.0 | 24±1.5 | 29±1.5 |

**Table 3.** Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of *C. distans* essential oil against *Candida* species

| Candida species | MIC (µg/mL) | MFC (µg/mL) |
|---|---|---|
| *C. parapsilosis* | 250 | 625 |
| *C. tropicalis* | 350 | 1000 |
| *C. kruseii* | 250 | 750 |
| *C. glabraata* | 500 | 750 |
| *C. albicans* | 750 | 1000 |

## Cytotoxic effect of *C. distans* essential oil against *C. albicans*

The antifungal activity of *C. distans* essential oil was further assessed by determining its effect on the viability of *C. albicans* cells using the MTT assay. Cell viability was evaluated after 24 and 48 h of treatment using MIC, MFC, 2×MIC, and 2×MFC concentrations of the essential oil.

As shown in Fig. 5, all tested concentrations significantly reduced the viability of *C. albicans* compared with the untreated control. After 24 h of treatment, the MIC concentration resulted in 49.6 ± 1.7% cell viability, whereas treatment with 2×MIC reduced viability to 31.7 ± 3.9%. Similarly, the MFC and 2×MFC concentrations reduced cell viability to 37.5 ± 1.8% and 13.5 ± 0.7%, respectively (Fig. 5).

A further reduction in cell viability was observed after 48 h of treatment. The MIC, 2×MIC, MFC, and 2×MFC concentrations exhibited cell viabilities of 14.3 ± 1.1%, 5.6 ± 6.7%, 4.8 ± 1.7%, and 1.2 ± 0.5%, respectively. Among the tested concentrations, the 2×MFC treatment produced the greatest reduction in fungal viability, demonstrating significant fungicidal activity following prolonged exposure (Fig. 5).

## Effect of *C. distans* essential oil on biofilm formation by *C. albicans*

The antibiofilm activity of *C. distans* essential oil against *C. albicans* was evaluated by quantifying biofilm formation after 24 and 48 h of treatment. As illustrated in Fig. 6, inhibition of biofilm formation increased with increasing essential oil concentration. After 24 h of treatment, the MIC (750 µg/mL), 2×MIC (1.5 mg/mL), MFC (1 mg/mL), and 2×MFC (2 mg/mL) concentrations inhibited biofilm formation by 12.4 ± 8.1%, 36.5 ± 2.4%, 25.9 ± 8.8%, and 56.2 ± 6.9%, respectively.

Extending the treatment period to 48 h further enhanced biofilm inhibition. The MIC, 2×MIC, MFC, and 2×MFC concentrations inhibited biofilm formation by 21.4 ± 11.0%, 58.6 ± 2.1%, 51.2 ± 2.1%, and 75.4 ± 4.8%, respectively (Fig. 7). Among all tested concentrations, the 2×MFC treatment exhibited the strongest antibiofilm activity at both time points. Overall, these findings demonstrate

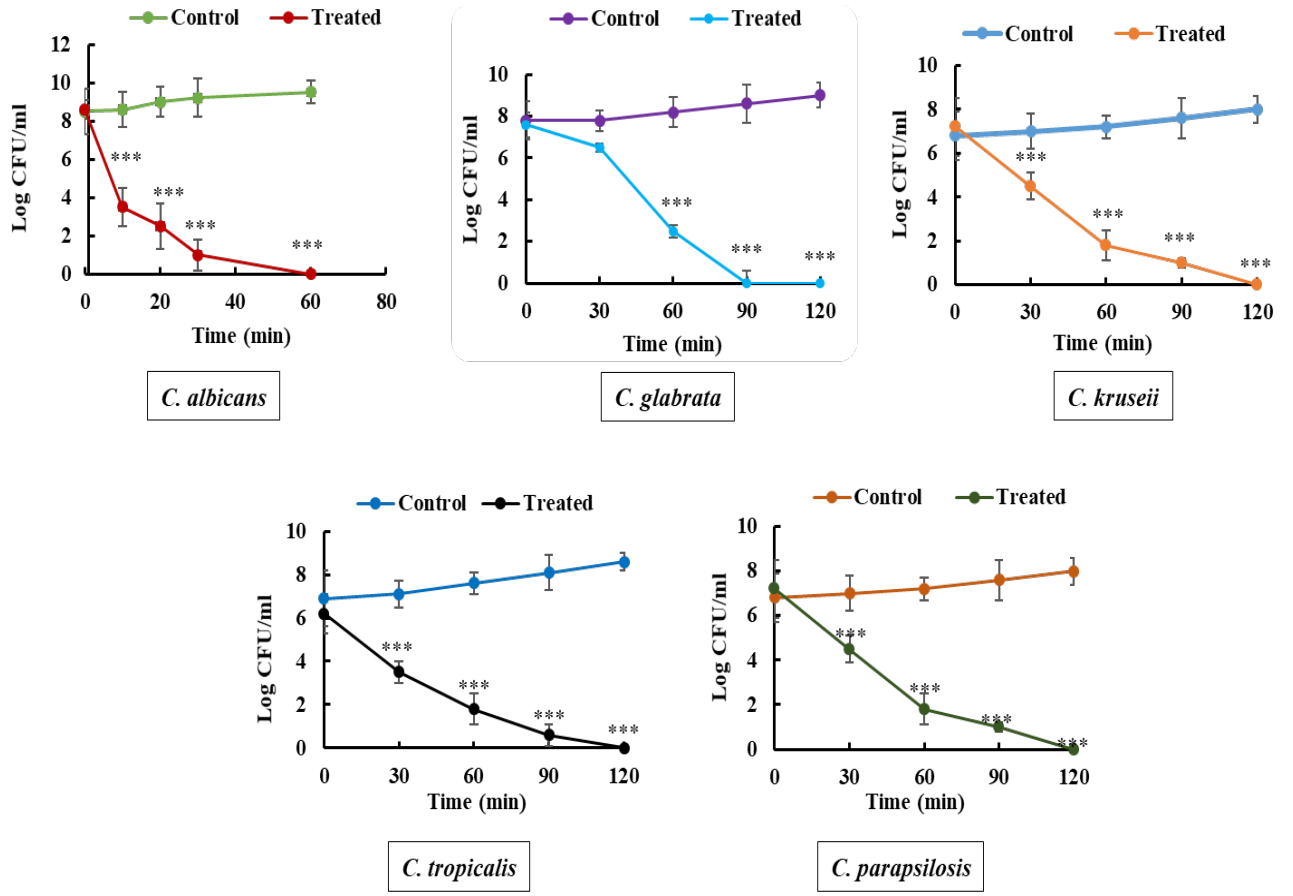


**Figure 2.** Determination of time dependent killing potential of *C. distans* essential oil against *Candida species*. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM). Statistical significance was calculated using Student t-tests. ***$p < 0.001$, compared to control

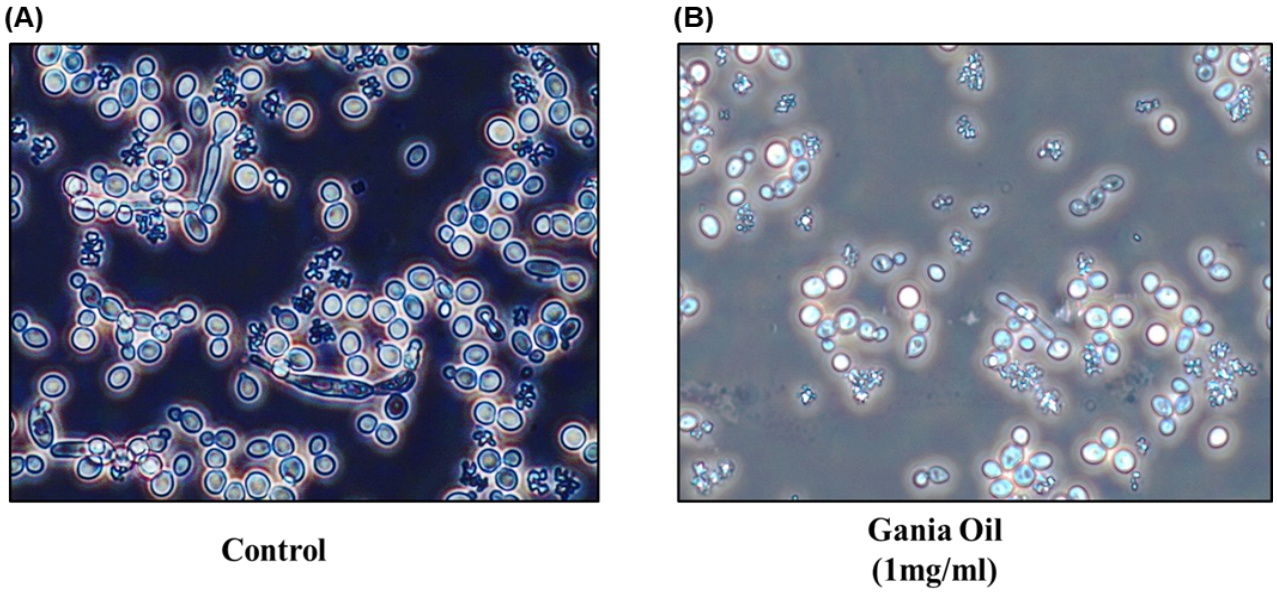


**Figure 3.** Effect of *C. distans* essential oil treatment on the morphology of *C. albicans*. (A) Control cells showing the normal morphology of the *C. albicans* with few budding yeast. (B) Treatment of *C. distans* essential oil caused the disruption of the *C. albicans* morphology with formation of debris. Images were taken under phase contrast microscope (Olympus, Japan) using 20X magnification

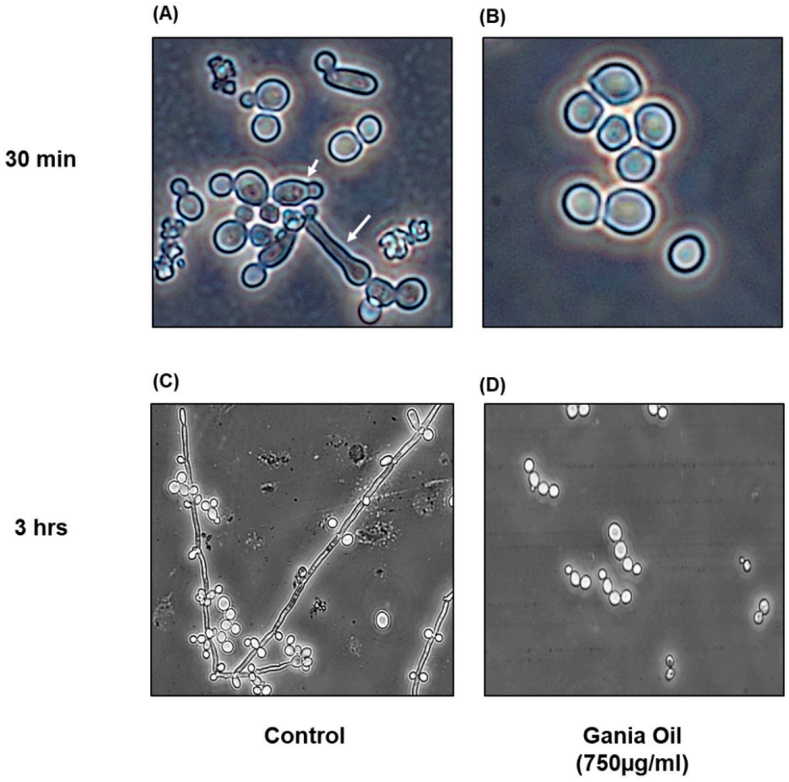


**Figure 4.** Germ tube test assay to determine the inhibitory potential of *C. distans* essential oil against yeast-to-hyphal transition of *C. albicans*. (A) Control cells showing an initial of the budding and development of hyphae within 30min of incubation (20X). (B) *C. distans* essential oil treatment inhibited the budding and hyphal growth in *C. albicans* cell (20X). (C) Yeast-to-hyphal transition can be seen in control cells within 3 hrs of incubation (10X). (D) *C. distans* treatment significantly inhibited the hyphal growth in *C. albicans* (10X). Images were taken under phase contrast microscope (Olympus, Japan)

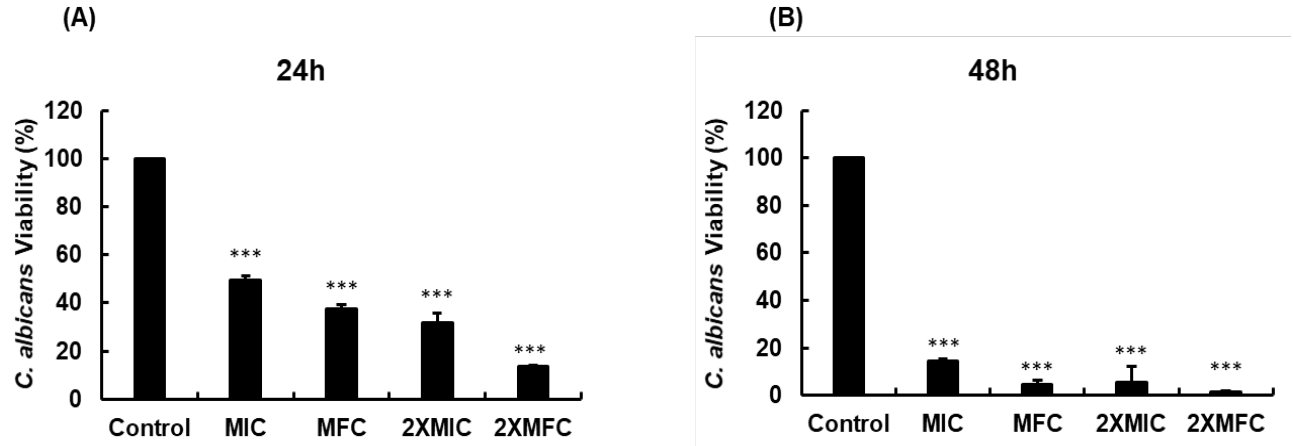


**Figure 5.** Effect of *C. distans* essential oil on cell viability of *C. albicans* determined by the MTT assay. (A) Cell viability at 24h. (B) Cell viability at 48h. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM). Statistical significance was calculated using Student t-tests. ***$p < 0.001$, compared to control

that *C. distans* essential oil effectively suppresses biofilm formation by *C. albicans* in a concentration- and time-dependent manner.

## Discussion

The genus *Cymbopogon* (family Poaceae) comprises approximately 144 species distributed throughout tropical and subtropical regions worldwide. Members of this genus are well recognized for their high essential oil content and diverse biological activities, resulting in their widespread use in the pharmaceutical, cosmetic, perfumery, and food industries[11,14]. Among these, *C. flexuosus* (lemongrass) has been extensively investigated and reported to possess antimicrobial, anti-inflammatory, antinociceptive, antimalarial, anti-obesity, and antihypertensive properties[3]. In contrast, *C. distans* remains comparatively underexplored despite its traditional medicinal importance. Previous studies have primarily focused on its chemical composition and antimicrobial activity against plant pathogens. For

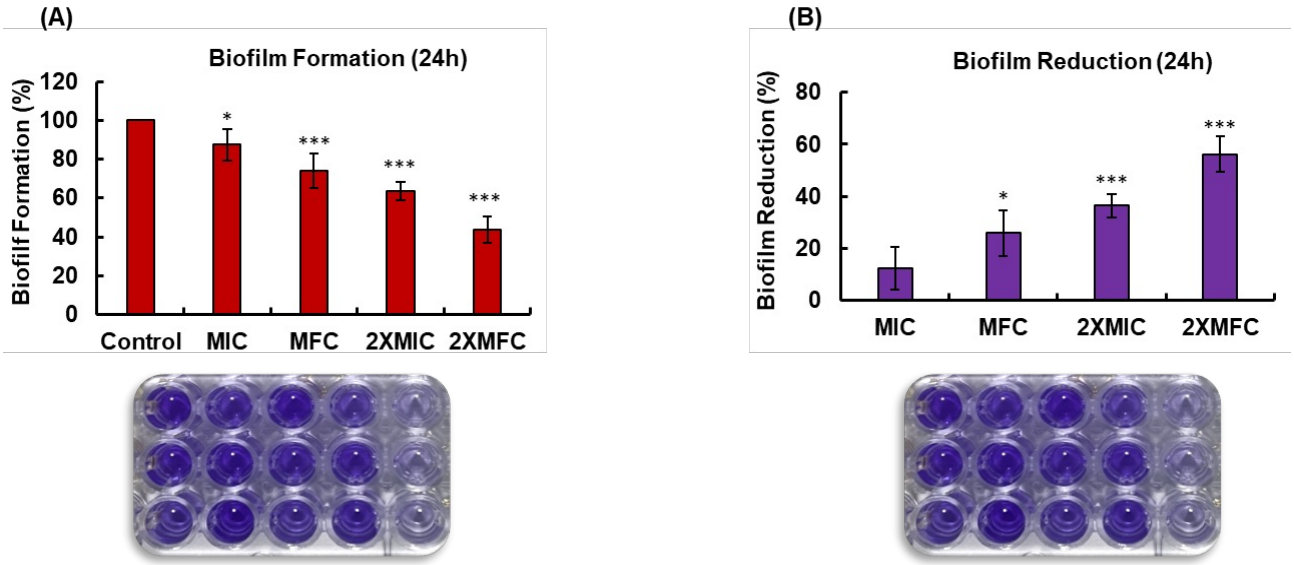


**Figure 6.** Effect of *C. distans* essential oil on biofilm formation *C. albicans* determined by the crystal violet assay. (A) Biofilm formation at 24h. (B) Biofilm reduction at 24h. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM). Statistical significance was calculated using Student t-tests. *$p < 0.05$, ***$p < 0.001$, compared to control

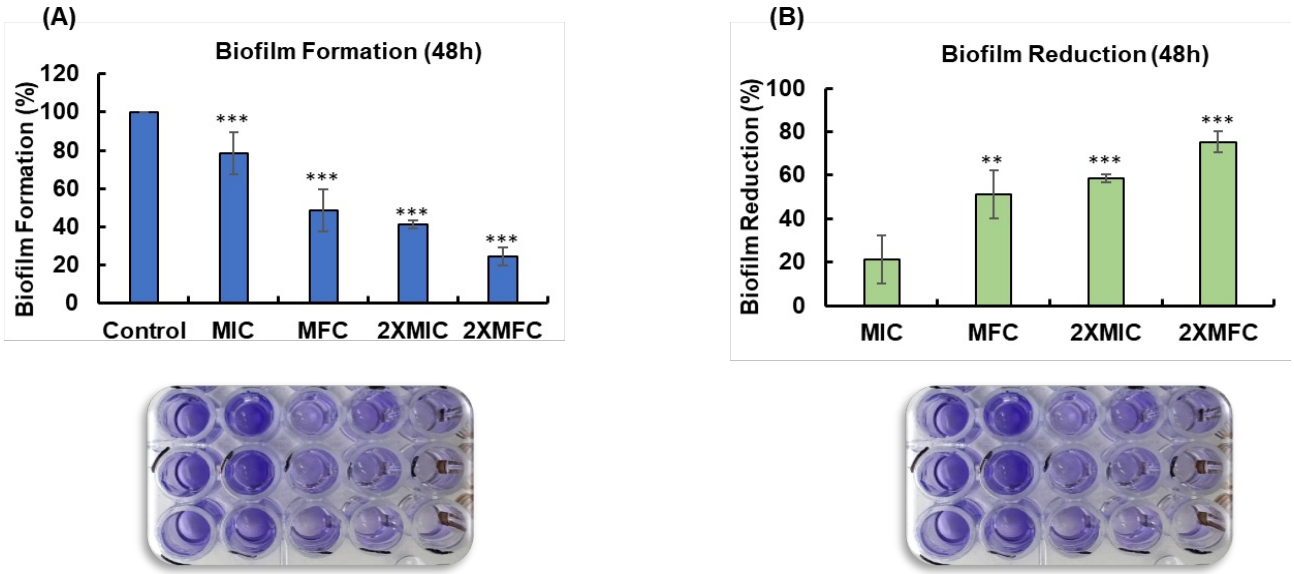


**Figure 7.** Effect of *C. distans* essential oil on biofilm formation *C. albicans* determined by the crystal violet assay. (A) Biofilm formation at 48h. (B) Biofilm reduction at 48h. Results are depicted as the mean of triplicate biological determinations ± standard error of the mean (SEM). Statistical significance was calculated using Student t-tests. *$p < 0.05$, ***$p < 0.001$, compared to control

example, Naik *et al*. reported significant antifungal activity of *C. distans* essential oil against several phytopathogenic fungi[18]. However, information regarding its activity against clinically important human fungal pathogens, particularly *Candida* species, remains limited. Therefore, the present study investigated the chemical composition, antifungal activity, inhibition of yeast-to-hyphal transition, and antibiofilm potential of *C. distans* essential oil against medically important *Candida* species.

GC–MS analysis revealed that camphene (25.40%) was the predominant constituent of *C. distans* essential oil, followed by D-limonene (17.95%), bornyl acetate (12.47%), neryl acetate (6.95%), α-pinene (6.68%), tricyclene (3.72%), and *p*-menth-1-en-8-ol (2.21%). These findings differ from those reported by Padalia *et al*.[19], who identified *p*-menthenols (24.5–26.7%) and δ-2-carene (19.9–28.2%)

as the major constituents of *C. distans* essential oil[19]. Such variations in essential oil composition are common and may result from differences in geographical origin, climatic conditions, altitude, harvesting season, and plant chemotype. Despite these compositional differences, the presence of monoterpenes and oxygenated monoterpenes in both studies supports the medicinal potential of *C. distans* essential oil.

Among the *Candida* species investigated, *C. albicans* remains the most clinically significant pathogen and is responsible for oral, cutaneous, vaginal, and systemic candidiasis[9,26]. Oral candidiasis is characterized by white or creamy-yellow plaques on the oral mucosa[20], whereas vulvovaginal candidiasis affects approximately 70% of women at least once during their lifetime, with recurrent infections occurring in nearly 8% of affected individuals[12]. In approximately 90% of these cases, *C. albicans* is the causative organism[12,20]. Although azoles, including fluconazole and itraconazole, remain the first-line therapeutic agents, their prolonged use has been associated with adverse effects and the emergence of antifungal resistance[8,21]. Consequently, there is increasing interest in identifying safer antifungal agents from natural sources.

The present study demonstrated that *C. distans* essential oil exhibited broad-spectrum antifungal activity against all tested *Candida* species. The essential oil produced concentration-dependent inhibition zones and showed low MIC and MFC values, indicating significant antifungal efficacy. The lowest MIC (250 µg/mL) and MFC (625 µg/mL) values were observed against *C. parapsilosis*, whereas *C. albicans* required comparatively higher concentrations (MIC, 750 µg/mL; MFC, 1 mg/mL). Time-kill analysis further demonstrated rapid fungicidal activity, with approximately a 4-log reduction in viable *C. albicans* cells within 10 min and complete killing achieved after 60 min. Complete fungicidal activity against the remaining *Candida* species was observed within 120 min. These findings suggest that *C. distans* essential oil possesses rapid and effective fungicidal activity against clinically relevant *Candida* species.

Our findings are consistent with those of Padalia *et al*.[19], who reported antifungal activity of *C. distans* essential oil against *C. albicans* with an MIC value of approximately 530 µg/mL[19]. Likewise, Taweechaisupapong *et al*.[27]. demonstrated that *C. citratus* essential oil exhibited significant antifungal activity against *C. albicans*, with MIC and MFC values ranging from 0.5 to 1.0 µL/mL[27]. The slight differences observed among studies may be attributed to variations in essential oil composition, extraction methods, fungal strains, and experimental conditions.

The pathogenicity of *C. albicans is* closely associated with its ability to undergo morphological transition from the yeast form to invasive hyphae. Hyphal development facilitates epithelial invasion, tissue penetration, and evasion of host immune responses, thereby contributing significantly to fungal virulence[5,28]. In the present study, treatment with *C. distans* essential oil caused pronounced morphological alterations and effectively inhibited germ tube formation, indicating suppression of the yeast-to-hyphal transition. These observations suggest that the essential oil not only inhibits fungal growth but may also attenuate an important virulence mechanism of *C. albicans*. Similar findings have been reported for several natural compounds, including disulfiram, prochlorperazine, trifluoperazine, CGS 12066B, magnolol, and honokiol, all of which inhibit hyphal development and reduce fungal pathogenicity[4,25].

Biofilm formation is another critical virulence factor of *C. albicans*, providing protection against host immune responses and markedly reducing susceptibility to conventional antifungal agents[5]. The present study demonstrated that *C. distans* essential oil significantly inhibited biofilm formation at all tested concentrations, with the greatest inhibition observed at the 2×MFC concentration after 48 h of treatment. The concentration-dependent reduction in biofilm formation suggests that the essential oil interferes with one or more stages of biofilm development. Comparable antibiofilm activity has previously been reported for magnolol and honokiol against *C. albicans* biofilms[25]. Collectively, these findings indicate that *C. distans* essential oil possesses multiple antifungal mechanisms, including direct fungicidal activity, inhibition of morphological transition, and suppression of biofilm formation.

Overall, the present findings demonstrate that *C. distans* essential oil exhibits promising antifungal activity against clinically important *Candida* species. The combined inhibition of fungal growth, yeast-to-hyphal transition, and biofilm formation suggests that this essential oil may serve as a promising natural source for the development of alternative antifungal formulations. Nevertheless, further investigations involving mechanistic studies, toxicity evaluation in mammalian cells, and *in vivo* efficacy studies are required before its clinical application can be considered.

## Conclusions

The present study demonstrates that *C. distans* essential oil possesses significant antifungal activity against clinically important *Candida* species, particularly *C. albicans*. GC–MS analysis revealed that the essential oil is rich in bioactive monoterpenes, with camphene, D-limonene, bornyl acetate, and neryl acetate as the major constituents. The essential oil exhibited significant concentration-dependent antifungal activity, effectively inhibited fungal growth, disrupted cellular morphology, suppressed the yeast-to-hyphal transition, and significantly reduced biofilm formation. These findings suggest that *C. distans* essential oil targets multiple virulence-associated

characteristics of *C. albicans*, highlighting its potential as a natural antifungal agent. Although further mechanistic investigations, safety evaluations in mammalian cells, and *in vivo* studies are required, the present findings provide a strong scientific basis for the future development of *C. distans* essential oil as a natural antifungal agent for the management of candidiasis.

## Limitations

The present study provides comprehensive *in vitro* evidence of the antifungal activity of *C. distans* essential oil against clinically important *Candida* species. However, several limitations should be acknowledged. First, the antifungal efficacy was evaluated only under *in vitro* conditions, and therefore the results may not fully reflect the complex interactions occurring during fungal infections *in vivo*. Second, although the essential oil demonstrated significant antifungal, anti-hyphal, and antibiofilm activities, the underlying molecular mechanisms responsible for these effects were not investigated. Future studies should focus on elucidating the molecular mechanisms of antifungal action, evaluating biocompatibility using mammalian cell lines, and validating the therapeutic efficacy of *C. distans* essential oil in suitable animal models of candidiasis before considering clinical applications.

## Declaration of AI use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

## Competing interests

Authors have declared that no competing interests exist.

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